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14. ABSTRACT Two approaches to control of microbiologically influenced corrosion (MIC) have been developed that do not require the use of biocides. These strategies include the following: 1) use of biofilms to inhibit or prevent corrosion and 2) manipulation (removal or addition) of an electron acceptor, including oxygen, sulfate and nitrate, to influence the microbial population.					
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NEW DEVELOPMENTS IN MITIGATION OF MICROBIOLOGICALLY INFLUENCED CORROSION

B. LITTLE, J. LEE & R. RAY

Naval Research Laboratory
Stennis Space Center, MS 39529-5004

SUMMARY: Two approaches to control of microbiologically influenced corrosion (MIC) have been developed that do not require the use of biocides. These strategies include the following: 1) use of biofilms to inhibit or prevent corrosion and 2) manipulation (removal or addition) of an electron acceptor, including oxygen, sulfate and nitrate, to influence the microbial population.

Keywords: Microbiologically influenced corrosion, biofilms, inhibition, electron acceptors

INTRODUCTION: The traditional approach to treating/controlling microbiologically influenced corrosion has been to use oxidizing (e.g. chlorine, bromine, ozone) or non-oxidizing (e.g. glutaraldehyde, carbamates, guanides, isothiazolines) biocides to reduce the numbers and types of organisms in a bulk medium. The problems with this approach are well documented. In some cases oxidizing biocides can cause corrosion. Neither oxidizing nor non-oxidizing biocides penetrate biofilms. Costerton et al.¹ reported that bacteria in biofilms were resistant to antibiotics and biocides at levels 500 to 5000 times higher than those required to kill planktonic cells of the same species. Persistent use of a single biocide treatment can allow more resistant microorganisms to develop and remain in the biofilm. Ridgway et al.² demonstrated that bacteria previously exposed to chlorine were more resistant than those never exposed. Resistance to a particular biocide can be overcome by periodically changing the biocide. Numerous investigators have observed a rapid resumption of biofouling at a time after biocide treatment or mechanical removal of cells from a surface. Regrowth or recovery may be due to the following: (1) Remaining viable cells reproduce a biofilm. (2) Residual biofilm imparts a surface roughness that enhances transport and sorption. (3) Oxidation of extracellular polymeric substances may provide nutrients for regrowth. The following sections will review two approaches to control of MIC that do not require the use of biocides.

Corrosion inhibition by biofilms

Corrosion inhibition due to the presence and activities of bacteria within biofilms has been reported for carbon steel,³⁻⁶ aluminum 2024,⁷⁻⁹ and copper.⁹ The mechanisms most frequently cited for corrosion inhibition by biofilms are as follows: (1) The biofilm forms a diffusion barrier to corrosion products that stifles metal dissolution. (2) Respiring aerobic microorganisms within the biofilm consume oxygen causing a diminution of that reactant at the metal surface. (3) Microorganisms produce metabolic products that act as corrosion inhibitors (e.g. siderophores). (4) Microorganisms produce specific antibiotics that prevent the proliferation of corrosion-causing organisms (e.g. sulfate-reducing bacteria [SRB]).

Several investigators have demonstrated that aerobic bacteria in a biofilm decreased the rate of corrosion of mild steel. Hernandez et al.⁴ observed increased corrosion resistance (R_p) when mild steel was exposed to a synthetic seawater medium (Vaananen nine-salt solution [VNSS]) containing *Pseudomonas* sp or *Serratia marcescens*. R_p values for the exposures (see Table I) demonstrate a marked increase in the presence of either bacterium. Higher R_p values for surfaces colonized by *S. marcescens* compared to those colonized by *Pseudomonas* sp. were attributed to higher numbers of *S. marcescens* cells attached to the metal surface. Impedance spectra (see Figure 1) after immersion in sterile and inoculated VNSS demonstrated the same basic trends indicated by the R_p data. The phase angle vs. frequency showed a maximum at 45° for sterile VNSS, indicating Warburg-type impedance and a diffusion-controlled reaction. In contrast, the maximum phase angle in bacterial suspensions was 75° and the modulus vs frequency indicated little corrosion during the first 20 days.

Hernandez et al.⁴ concluded the following:

- (1) Corrosion inhibition required bacterial adhesion.
- (2) The inhibition effect disappeared when *in situ* cells were fixed in glutaraldehyde.
- (3) When cell-covered carbon steel surfaces were transferred to nutrient-deficient synthetic seawater, the inhibition continued despite the predicted diminished respiration.

(4) After exposure to natural seawater the inhibitive effect disappeared and *Pseudomonas* could not be located in the biofilm.

(5) The *Pseudomonas* sp. system was contaminated after 20 days.

Jayaraman et al.³ designed experiments to investigate whether or not corrosion inhibition by aerobic biofilms was a general phenomenon. They used carbon steel coupons exposed in a complex liquid medium (Luria-Bertani [LB] broth) and a synthetic seawater (VNSS) and fifteen different pure-culture bacterial suspensions representing seven genera. Compared to sterile controls, the mass loss in the presence of bacteria decreased by 2-15 fold. Corrosion inhibition varied among the genera and the extent of corrosion inhibition depended on the nature of the biofilm: an increased proportion of live cells decreased corrosion. The authors concluded that a thin layer of actively respiring cells was required to inhibit corrosion. When cells were killed with an addition of an antibiotic to the medium it caused an immediate increase in the corrosion.

Eashwar and Maruthamuthu¹⁰ reviewed the literature on ennoblement (a positive shift in open-circuit potential [E_{corr}]) of metals in marine environments. They concluded that ennoblement is regulated by metabolic activity rather than the physical presence of microorganisms in a biofilm and hypothesized that a strengthening of the passive film on stainless steel alloys is siderophore-assisted. Siderophores are iron-chelators formed by

bacteria at near neutral pH. According to Eashwar and Maruthamuthu¹⁰ a range of ennoblement is possible due to alloy-siderophore compatibility, i.e., an intrinsic property of the alloy to profit from the presence of the inhibitor.

Jayaraman et al.¹¹ used genetically engineered biofilms in a complex, nutrient-rich medium (modified Baar's) to produce antimicrobials specific for SRB. They calculated a 50-fold reduction in the corrosion rate of 304 stainless steel when SRB growth was inhibited by *in situ* production of gramicidin. Similar results were obtained with Society of Automotive Engineers (SAE) 1080 mild steel. Ömek et al.¹² demonstrated that pitting attack of aluminum 2024 in LB medium was greatly reduced by the anionic peptides, polyaspartate and polyglutamate secreted by genetically engineered and natural *Bacillus subtilis* and *B. licheniformis*, respectively. Formation of an aluminum/polyaspartate or polyglutamate complex may have reduced the uniform corrosion rate of aluminum.

Table I

R_p of mild steel ($K\Omega\text{-cm}^2$) after different times of exposure to NSS with and without bacteria (4×10^8 cells/mL)^a

	Exposure Time (days)		
	10	20	30
<i>Pseudomonas</i> sp. S9	28 ± 3	(B)	(B)
<i>S. marcescens</i>	35 ± 5	34 ± 3	15 ± 2
Sterile NSS	5.0 ± 0.5	4 ± 0.5	3.5 ± 0.5

^(a) Results correspond to a mean value obtained on 5 specimens ± the standard deviation.

^(b) The system was contaminated after 20 days.

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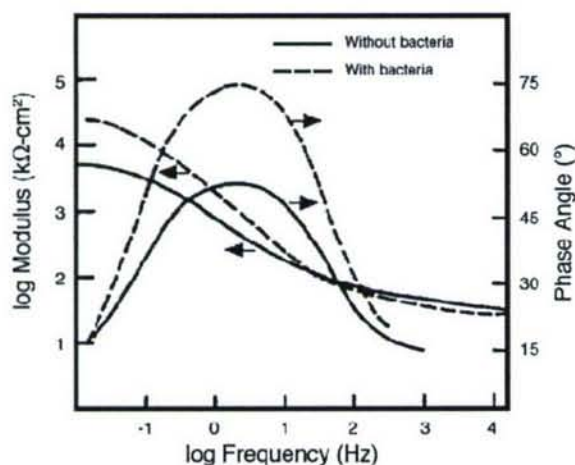


Figure 1. EIS spectra obtained after 20 days of exposure with and without *Pseudomonas* sp. S9. Curves are given for individual runs. Standard deviation between replicates was ± 10 percent.⁴
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Despite the laboratory studies indicating possibilities for using bacteria to inhibit corrosion of a number of alloys, applications have not been totally successful. Arps et al.¹³ evaluated the concept of corrosion control using regenerative biofilms at Three Mile Island Nuclear Power Station, Harrisburg, PA. The field experiment was conducted using a consortium of five bacteria (one polymyxin-producing bacillus strain and four gramicidin S producing bacillus strains) to inoculate service water in a sidestream containing coupons of 304 stainless steel, 1018 carbon steel, and cartridge brass. Their results indicate that the inhibition of corrosion by the consortium was small (see Figure 2). Pitting of brass specimens was noted in the presence or absence of the consortium.

The same organisms and mechanisms to which MIC has been attributed have also reportedly inhibited corrosion. For example, *Pseudomonas* and *Serratia* are reported to increase the corrosion rate of iron and nickel compared to sterile conditions.¹⁴ The same researchers have shown that *Pseudomonas* and *Serratia* can have a protective effect on some metals under some circumstances.^{5,6} Metal-binding by extracellular polymers has been reported as a mechanism for both MIC¹⁵ and for corrosion inhibition.¹⁶

Jigletsova et al.¹⁷ and Rodin et al.¹⁸ examined the influence of environmental conditions on corrosion inhibition by biofilms and demonstrated that the division of bacteria into ones that caused corrosion and ones that inhibited corrosion was entirely arbitrary and that the corrosive properties of biofilms varied with culture conditions, especially with culture medium composition. Jigletsova et al.¹⁷ used mild steel coupons exposed to a natural consortium of bacteria, including oil-oxidizing aerobes and sulfate-reducing bacteria isolated from oil-processing waters. During biofilm formation in glucose-mineral medium with peptone, corrosion losses increased vs. sterile control. However corrosion decreased when coupons with biofilms were transferred into enriched LB medium (see Figure 3a). In a separate experiment, an increase in corrosion was observed when coupons were transferred from LB to the minimal medium (see Figure 3b). Their data indicate that environmental conditions determine the specific microbiological effect on corrosion processes – not the individual organisms.

Webster and Newman¹⁹ examined the impact of media constituents on localized corrosion and made the following observations: localized corrosion would not readily occur unless chloride (Cl^-) was the predominant anion in the medium. They concluded that Cl^- must be present in a concentration at least comparable to that of all other anions combined, otherwise corrosion was inhibited even at high H_2S concentrations up to 500 ppm. Reduction of the ratio of Cl^- to other anions increased the time to initiation and decreased the rate of propagation of the corrosion. Other corrosion investigators have concluded that extra nutrients cannot be added to stimulate bacterial growth if those nutrients inhibit corrosion by adding too many non-chloride ions.²⁰ Anions, including sulfate, hydroxide, phosphate, acetate, carbonate and nitrate can inhibit pitting corrosion. It is possible that bacterial consumption and fixation of nutrients, including sulfate could render an initially inhibiting solution aggressive by removing non-chloride ions.

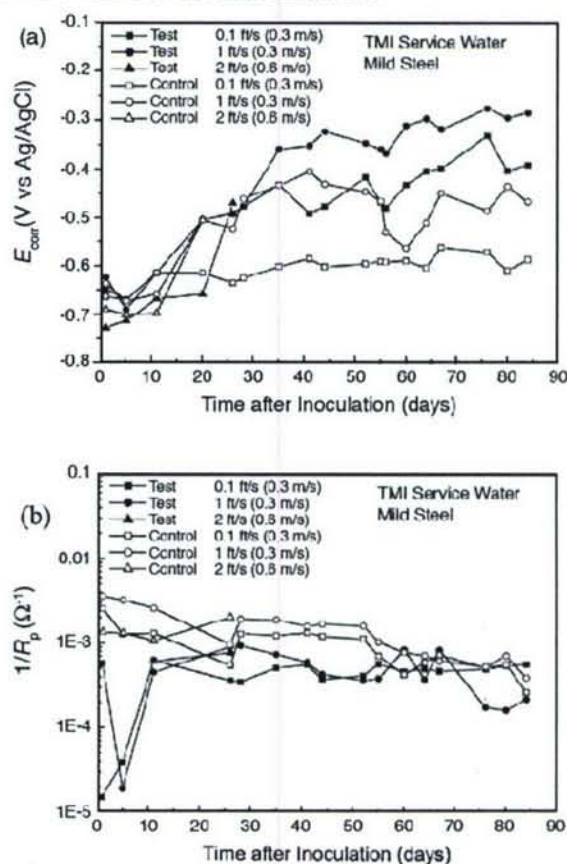


Figure 2. Time dependence of (a) E_{corr} and (b) $1/R_p$ for mild steel disc specimens exposed to a CCURB bacterial consortium containing five strains. The test (\blacktriangle) and control (\triangle) specimens at 2 ft/s were monitored for about 3 weeks before the monitoring cables were switched to brass specimens.¹² © NACE International 2003

An additional complication in the interpretation of the laboratory studies on biofilm inhibition of corrosion is the effect of culture media on electrochemical measurements and on corrosion reactions. Most of the laboratory studies on corrosion inhibition have been conducted in laboratories with nutrient-rich media. The impact of nutrients on electrochemical measurements and MIC was investigated by Webster and Newman.¹⁹ They observed interferences in electrochemical measurements when yeast extract was included in the culture medium/electrolyte. The interferences were removed when the yeast extract was removed. All of the nutrient-rich media used in the cited laboratory studies on corrosion inhibition contained yeast extract. Modified Baar's medium contains 10 gL⁻¹ tryptone, 5 gL⁻¹ yeast extract, and 10 gL⁻¹ NaCl. The LB medium contains 10 gL⁻¹ tryptone, 5 gL⁻¹ yeast extracts and 10 gL⁻¹ NaCl. VNSS consists of 1.0 g peptone, 0.5 g glucose, 0.5 g starch, 0.5 g yeast extract, 0.01 g hydrated ferrous sulfate, 0.01 g NaHPO₄ and 1,000 mL of a nine-salt solution, containing 17 g sodium chloride. The cited studies on corrosion inhibition in complex media have compared corrosion in sterile and inoculated media, but none have carefully studied the impact of media constituents on the electrochemical parameters used to quantify corrosion.

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Alter potential electron acceptors to inhibit specific groups of bacteria

Both removal and addition of electron acceptors has been used as a means of controlling microbial populations and MIC in seawater injection systems where seawater is injected into oil reservoirs to maintain pressure. In these applications, oxygen is removed to minimize corrosion. However, in the anaerobic environment, growth of SRB is encouraged and corrosion of iron and steel alloys is the result. The concentration of sulfate in seawater is >2.0 gms/l. Rizk et al.²¹ used nanofiltration to reduce sulfate in seawater from 2.6 grams/liter to 50 mg/l. In laboratory studies they were able to demonstrate that decreased sulfate concentration in injected seawater significantly restricted the activity of SRB and the amount of hydrogen sulfide decreased as a direct function of the amount of sulfate in the water. In contrast, Jhobalia et al.²² demonstrated that high sulfate concentration in the medium (increases from 1.93g/l to 6.5 g/l) could inhibit growth of SRB and the corrosion rate of mild steel.

Laboratory and field experiments have demonstrated that nitrate treatment can be an effective alternative to biocide treatment to reduce the numbers of SRB and their activity, a process known as biocompetitive exclusion. The addition of nitrate can induce a shift in the dominant population from SRB to nitrate-reducing bacteria (NRB). Nitrate treatment was implemented on an oil platform in the North Sea (Veslefrikk).²³ The change from glutaraldehyde treatment to nitrate resulted in a dramatic change in the bacterial community. The SRB population decreased and the nitrate-reducing bacteria (NRB) increased. After four months of nitrate addition the activity of SRB in the biofilm was markedly reduced as measured with respiratory methods and an enrichment of NRB was measured. After 323 months of nitrate treatment, SRB numbers were reduced 20,000 fold and SRB activity was reduced 50 fold. Corrosion measurements decreased from 0.7mm/year to 0.2 mm/year. Gullfaks platforms²⁴ have been treated with nitrate to reduce H₂S production. 1000-fold reduction in SRB numbers and a 10-20-fold reduction in sulfate respiration activity and a 50% reduction in corrosion. Reservoir characteristics and nutrient availability have a significant impact on the effectiveness of nitrate injection.

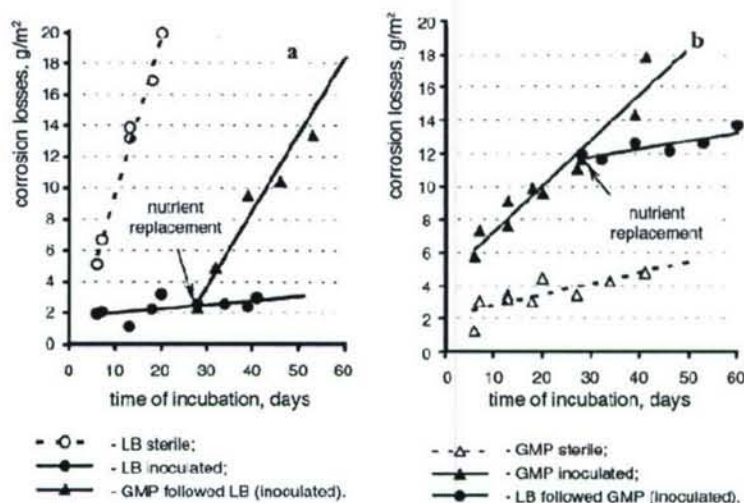


Figure 3. The dynamics of corrosion losses with nutrient replacement: (a) the biofilm grown in LB was transferred into GMP; (b) the biofilm grown in GMP was transferred into LB medium.¹⁷ © NACE International 2005

Hubert et al.²⁵ demonstrated that both nitrate and nitrite are effective treatments for decreasing sulfide concentrations. The required dose depends on the concentration of oil organics used as the energy source by the microbial community. Because of its higher oxidative power, nitrate can remove more oxidizable oil organics than nitrite. However, nitrite is a strong inhibitor of SRB.

Both the addition and removal of oxygen have been proposed as corrosion control measures. Khanal and Huang²⁶ demonstrated that oxygenation was effective in controlling sulfides during anaerobic treatment of high-sulfate wastewater. However, SRB in biofilms depend on other organisms to remove oxygen and produce nutrients, so they can survive in aerated systems. Furthermore, oxygen exacerbates the problem of corrosion. Hamilton²⁷ proposed a model for MIC in which he concluded that several MIC mechanisms involved a process of electron transfers from base metal to oxygen as the ultimate electron acceptor through a series of coupled reactions. The specific coupled reactions varied with mechanism and causative organism. In the case of SRB, sulfate, an intermediate electron acceptor, is reduced to sulfide that reacts with a metal to form a corrosion product that ultimately transfers electrons to oxygen. Consistent with that model, most reported cases of SRB induced corrosion are in environments with some dissolved oxygen in the bulk medium.^{28,29}

Removing oxygen from seawater has been proposed as a corrosion control measure for unprotected carbon steel ballast tanks. Matsuda et al.³⁰ conducted shipboard trials by sealing a ballast tank at the deck and installing vertical pipes into the headspace. They reported that pumping pure nitrogen gas into the headspace for 1.5 hr reduced oxygen levels in the seawater to approximately 0.2 mg/L and decreased the rate of uniform corrosion of carbon steel by 90% as determined by weight loss. However, in laboratory experiments, Lee et al.³¹ compared corrosion resulting from stagnant aerobic natural seawater with corrosion resulting from stagnant anaerobic natural seawater over a one-year period. They demonstrated the following: (1) corrosion was more aggressive under totally anaerobic conditions as measured by instantaneous corrosion rates ($1/R_p$) and weight loss, (2) under aerobic conditions corrosion was uniform and the surface was covered with iron oxides (lepidocrocite and goethite) and (3) under anaerobic conditions the corrosion was localized pitting and the corrosion products were mackinawite and pyrrhotite. Lee et al.³² designed field experiment to evaluate deoxygenation of natural seawater as a corrosion control measure for unprotected carbon steel seawater ballast tanks. They demonstrated the difficulty of maintaining hypoxic seawater. Using a gas mixture it was possible to displace dissolved oxygen. However, aerobic respiration and corrosion reactions consumed oxygen and produced totally anaerobic conditions within the first days of hypoxia. When gaskets and seals failed, oxygen was inadvertently introduced. The impact of oxygen ingress on corrosion depends on the amount of oxygen in the system at the time oxygen is introduced. Carbon steel exposed to cycles of hypoxic seawater and oxygenated atmosphere had higher corrosion rates than coupons exposed to cycles of either consistently aerobic or deoxygenated conditions.

CONCLUSIONS: It is difficult to compare the laboratory studies on corrosion inhibition due to biofilms because of the differing experimental conditions, organisms and culture conditions. However, to-date there has been no successful field application of this strategy to control MIC. The following critical issues must be addressed before bacteria can be used to predictably inhibit corrosion: (1) the stochastic nature of biofilms and (2) contamination and/or natural competition. One of the fundamental assumptions in much of the work on corrosion inhibition by biofilms is that biofilm formation is predictable and controllable. Microorganisms colonize all engineering materials, but there is a stochastic nature to areal coverage and thickness that has never been successfully modeled. Bacteria in pure cultures or in consortia do not form uniform, predictable biofilms. Growth rate depends on substratum, available nutrients, temperature and electron acceptors. Cells within biofilms die and can cause aggressive corrosion. Clumps of cells can slough from the surface transforming a homogeneous biofilm to a patchy one. Furthermore, biofilm composition is affected by small perturbations in the environment (e.g. temperature, nutrient concentration, and flow). The response of microorganisms within biofilm cannot be predicted with certainty. Natural competition with extraneous organisms can alter the microbial constituents of a biofilm. Hernandez et al.⁴ observed contamination of controls after brief experiments (20 days) and a change in microbial composition of biofilms after introduction into a natural system. The bigger problem is that most investigators do not evaluate the microbial population at the end of the experiment and have no insight into possible contamination or changes in the engineered biofilm.

Controlling microbial corrosion by controlling the electron acceptor has been used successfully in limited applications in the seawater injection systems. The long-term consequences are not clear. Nitrate/nitrite supplementation is a new technology and some work is underway to carefully characterize the bacteria that are developing in nitrate-rich water. NRB reduce nitrate to N_2 with several possible intermediates, including

nitrite. There are several potential mechanisms for the observed inhibition of SRB due to addition of nitrate. One of them is competition for carbon sources. When competing for the same carbon source, NRB out-compete SRB because nitrate is a stronger oxidizer than sulfate. This argument is valid only in carbon-limited waters. Toxic reaction products from the reduction of nitrate to N_2 may inhibit SRB. A shift in the redox potential in the system may also inhibit SRB. As a consequence of nitrate reduction, the redox potential will likely increase, producing unfavorable conditions for sulfate reduction.

Biocides control MIC by decreasing the microbial population, whereas control by nitrate addition relies on a population of nitrate-utilizing bacteria in the system. Hubert et al.³³ suggested that bioaugmentation, in which *ex situ* grown microorganisms could be injected with the nitrate if indigenous NRB were lacking. Despite the possibility of bioaugmentation, there are several reports of failures. Bouchez et al.³⁴ attempted to inoculate a nitrifying sequencing batch reactor with an aerobic denitrifying bacterium. The added bacterium disappeared after two days. Similarly, Hubert et al (2004) reported that introduction of microorganisms into natural communities is difficult.

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